[i]

Cells in 50 µl media (cells plated the night before)

The next day the plate is left on ice for loading CPA

Add 50 µI 0.50M mannitol to all wells

Remove mannitol/media

Wash wells with 100 μ l of final CPA concentration

Add 100 µl of final CPA concentration to appropriate wells

Cool plate at -1.0 °C/min. to -80 °C

Leave plate at -135 °C overnight

Plate is removed from freezer and warmed to ~4 °C; 150 μ I 0.5M mannitol/media (warmed to 37 °C) is added during thawing

Put the plate on ice and remove mannitol/CPA

Wash wells 2x with 100 µl 0.5M mannitol

Wash wells 2x with 100 µl DMEM (10%FCS)

Leave 200 μl DMEM (10%FCS) in well for Alamar Blue assay

FIG. 1

Hand Hand County House Street Hand White Street

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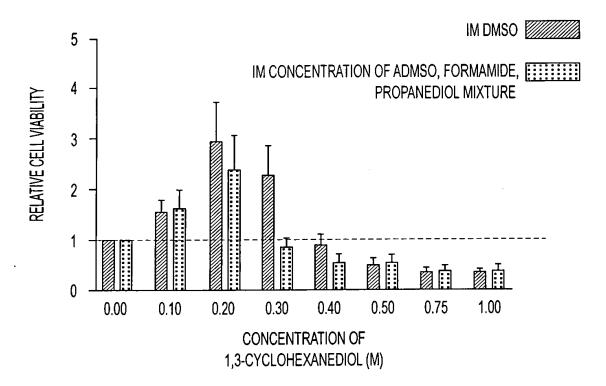


FIG. 2

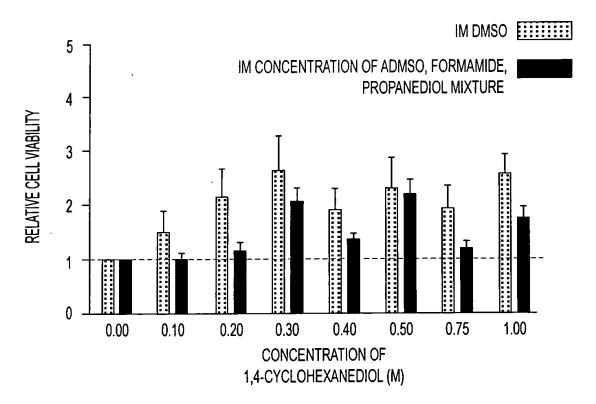


FIG. 3

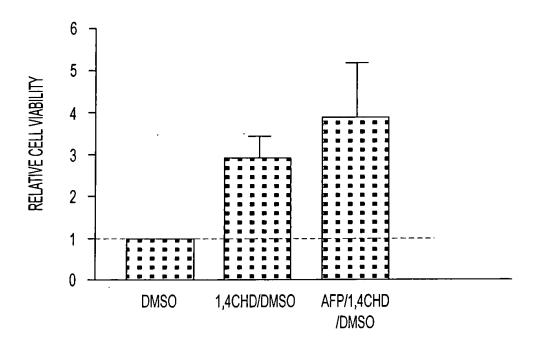


FIG. 4

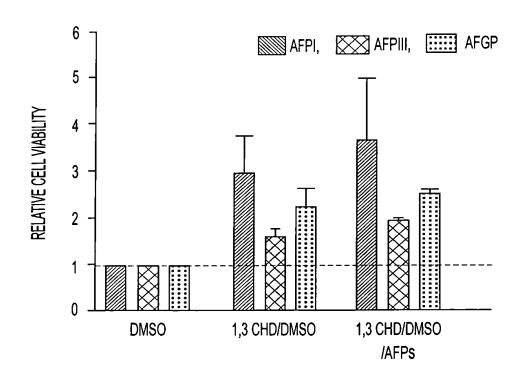


FIG. 5